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TITLE: The Effects of Signaling Through the EGF Receptor System Upon Regulation of Growth in Human Mammary Epithelial Cells

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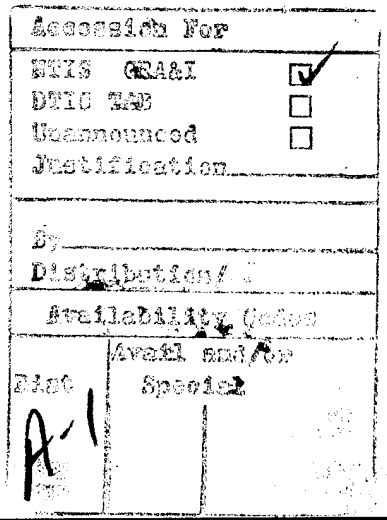

Becky Mottsdale 5/31/95
PI Signature Date

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Introduction

Overexpression of the human EGF receptor (HER1) and HER2 are associated with human breast cancer. This aim of this proposal is to explore the relationship between misregulation of signaling through these receptors and the malignant transformation of human mammary epithelial cells in an *in vitro* cell culture system. Two methods of growing these cells in a physiologically relevant manner is on semipermeable membranes of on a synthetic basement membrane.These culture systems allow the formation of polarized epithelial cells. On the basement membrane these structures resemble the alveoli and connecting ducts found in the mature mammary gland. The intracellular trafficking patterns of the EGF receptor play an important role in attenuation of the mitogenic signal transduced through this receptor. I will determine if perturbing the intracellular trafficking of the EGF and/or HER2 results in abnormal growth of human mammary epithelial cells in culture.

First, I will define the expression levels of HER1 and HER2 in normal human mammary epithelial cells by Western Blot and RT-PCR. Additionally, I will determine the role of trafficking in regulating the half life of these receptors by standard pulse chase procedures. Finally, these normal trafficking patterns will be altered either by introducing an EGF receptor trafficking mutant or by overexpressing the HER1 or HER2. The regulation of growth and organization in cells misregulating the EGF receptor signaling system will be assessed by proliferation assays and microscopy.

Body

The levels of the Human EGF Receptor (HER1) and the related receptors HER2,3 and 4 were examined in human mammary epithelial cells (HMEC's) by Western blot. When HMEC's are grown on plastic tissue culture dishes HER1 is expressed at high levels, while HER2 and HER4 are only expressed at low levels. No HER3 was detected. Interestingly, the level of HER1 decreased dramatically when grown on semipermeable membranes, or on a synthetic basement membrane. Unexpectedly, the level of HER2 also decreased, but only 3-4 fold as compared to 10 fold downregulation for HER1. The level of HER4 was low whether the cells were cultured on plastic or on membranes; however, on plastic HER4 protein runs as a doublet, while on membranes only the faster mobility band is expressed. (See figure 1)

The expression of the various ligands to these receptors was examined by RT PCR, by others in the laboratory. HMEC's express high levels of amphiregulin; moderate levels of TGF-alpha, Heparin binding (HB) EGF; and very low levels of EGF and ß cellulin. There is no known ligand for HER2, but heregulin is a ligand for both HER3 and HER4 which heterodimerize
with HER2. RT PCR demonstrates that the HMEC cells express significant levels of heregulin. (See figure 2)

The effects of HER1 stimulation on the levels of HER2 were examined in both HMEC's and mouse fibroblasts. Addition of exogenous EGF leads to a decrease in the levels of HER2 as assessed by Western blot. This downregulation occurs in 3 hours, thus, the mechanism appears to be post transcriptional. Since our laboratory has mouse fibroblast cell lines containing many mutants of the HER1, I have pursued the mechanism of HER1 stimulation on HER2 trafficking in the fibroblast cell line. A HER1 construct harboring a point mutation in the kinase domain, rendering it kinase inactive. Interestingly, stimulation of this HER1 mutant with exogenous EGF also leads to a downregulation in the level of HER2. (See figure 3)

Conclusions

1. HER expression levels:

a. HER1 is expressed at high levels in HMEC growing on plastic, but is dramatically reduced upon culturing on membranes or a synthetic basement membrane.

b. HER2 is expressed at a low level in HMEC growing on plastic, and its expression is further reduced by culturing on membranes.

c. HER3 could not be detected in HMEC grown on plastic or on a membrane.

d. HER4 is expressed at a low level in HMEC cultured on plastic or membranes. Interestingly, it is expressed as a doublet on plastic, but only a single band when grown on membranes.

2. HER ligand expression:

a. Several HER1 ligands are expressed in HMEC's: Amphiregulin is abundant, TGF-alpha and HB EGF are moderately expressed, and EGF and ß Cellulin are rare.

b. The ligand for HER3 and HER4, heregulin, is expressed at significant levels in HMEC's.

3. Effects of HER1 stimulation on the trafficking of HER2:

a. Addition of exogenous EGF to stimulate HER1 results in the downregulation of HER2 levels.

b. This downregulation occurs within 3 hours and is thought to be post transcriptional.

c. A kinase negative mutant of HER1 retains the ability to downregulate HER2 in response to EGF.

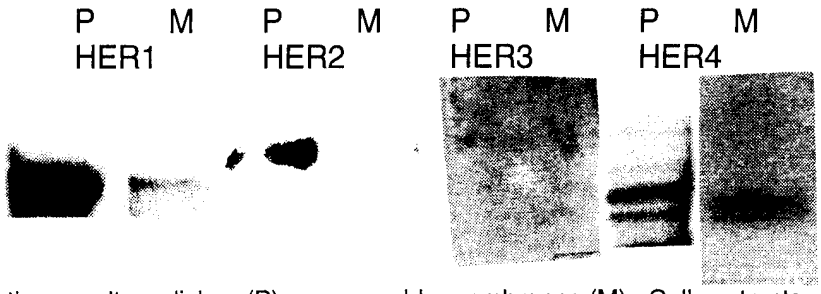
Four mechanisms by which EGF stimulation of HER1 could lead to a downregulation in the level of HER2:

1. Heterodimerization followed by co-internalization
2. Transphosphorlation of HER2 by HER1, leading to HER2 activation and internalization
3. Heterodimerization directly activated HER2, leading to its internalization
4. Signaling through HER1 leads to transcriptional repression of HER2 mRNA

The fourth mechanism can be ruled out by the time course shows that the downregulation occurs within 3 hours. The second mechanism can also be ruled out because a kinase negative version of HER1 is capable of reducing HER2 levels in response to EGF. Distinguishing between mechanisms 1 and 3 can be differentiated by determining if a HER1 mutant which lacks the whole cytoplasmic tail of the receptor can downregulate the levels of HER2 in response to EGF. This truncation mutant of HER1 is unable to be internalized in response to EGF, yet is reported to retain the ability to heterodimerize with HER2. Thus, if this truncated receptor can reduce the levels of HER2, then the two receptors are not co-internalized and heterodimerization can directly activate HER2, leading to its internalization.

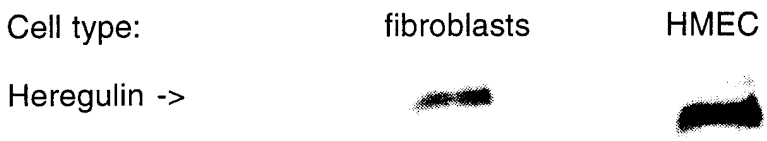
Appenidix

Figure 1: Western blot showin expression levels of HER's in HMEC's



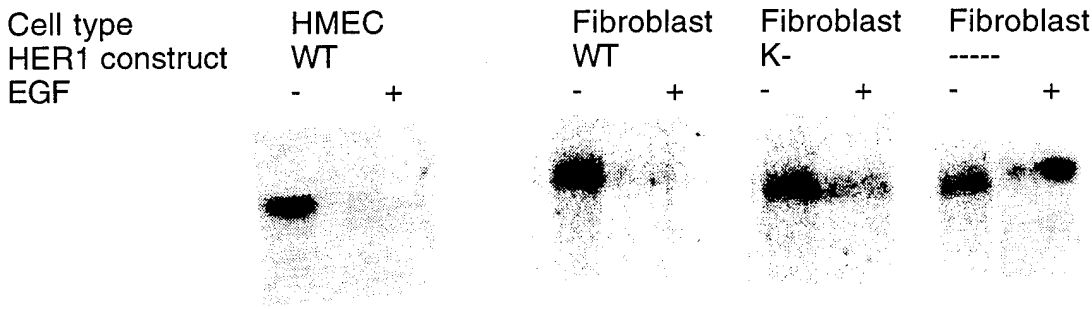
HMEC's were cultured for 7 days on plastic tissue culture dishes (P) or permeable membranes (M). Cells extracts were separated on SDS PAGE and Western blotted for the various receptors.

Figure 2: RT PCR analysis of Heregulin expression in HMEC's



RNA was isolated from HMEC's and mouse fibroblasts using TRI REAGENT. The RNA was then subjected to RT PCR in the presence of 32P-dCTP using primers specific to heregulin. The resulting products were analyzed by autoradiography.

Figure 3: Western blot analysis of HER2 levels in response to EGF



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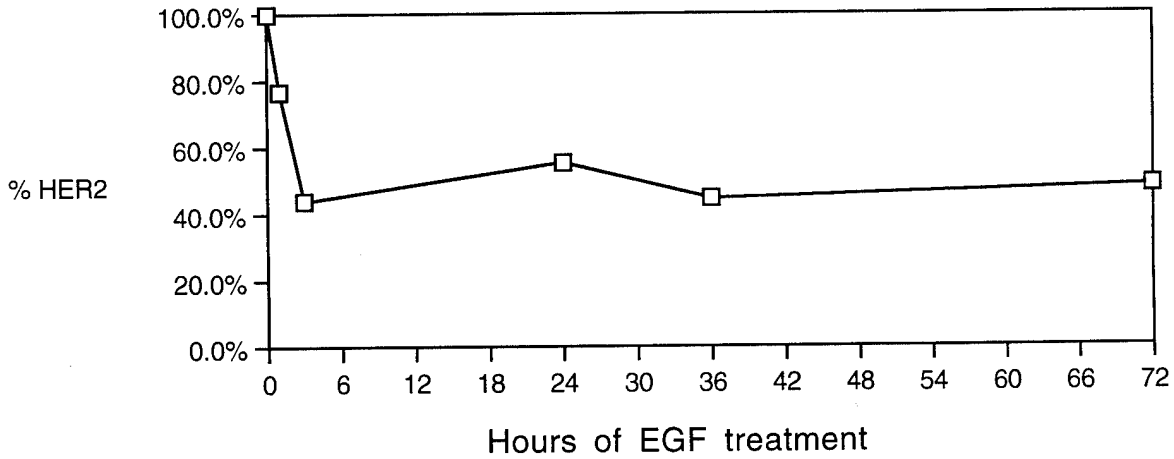

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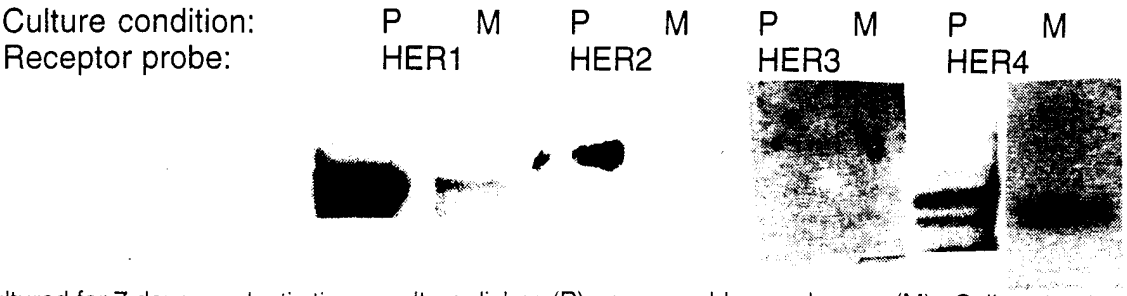

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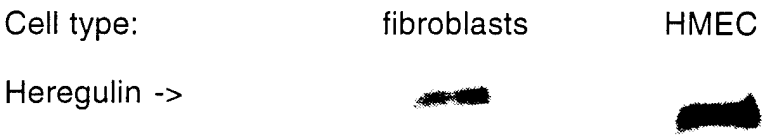

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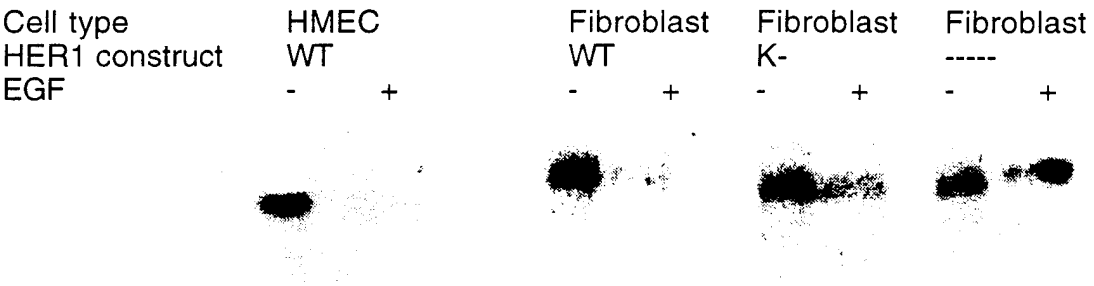

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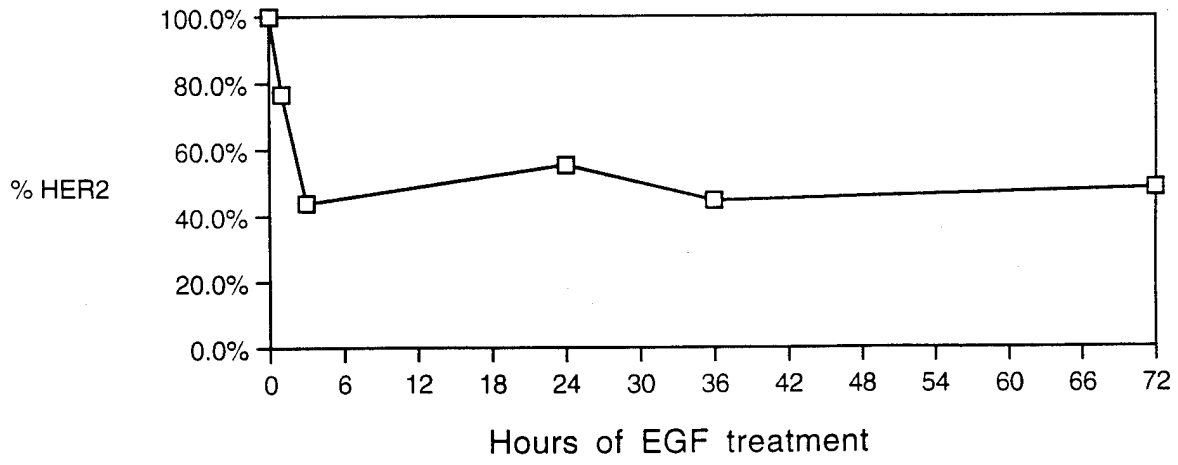

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